Supporting Information

Hybrid Vesicles as Intracellular Reactive Oxygen Species and Nitric Oxide Generators

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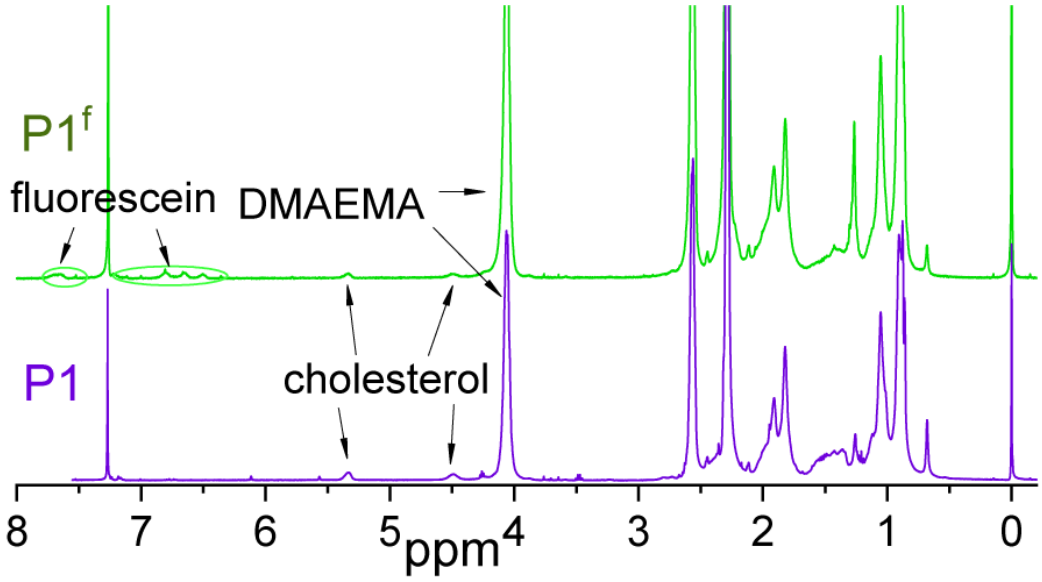

Figure S1. ^{1}H NMR (400 MHz) spectra of the block copolymers P1 and ^{f}P1 in $CDCl_3$.

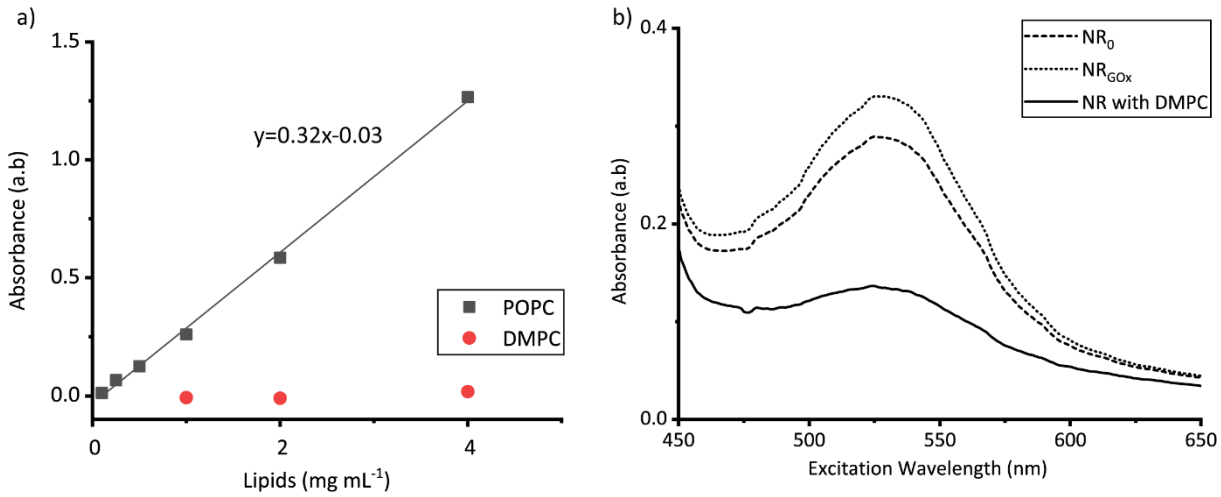

Figure S2. Lipid concentration determined by the sulfo-phospho-vanillin reaction. a) Typical concentration curve for (non-extruded) POPC liposomes including the linear fit used to determine unknown lipid amounts in samples, compared to (non-extruded) DMPC liposomes. b) Absorbance spectra of sulfo-phospho-vanillin reacted NR_0, NR_{GOx} and NR containing DMPC instead of POPC after size exclusion chromatography.

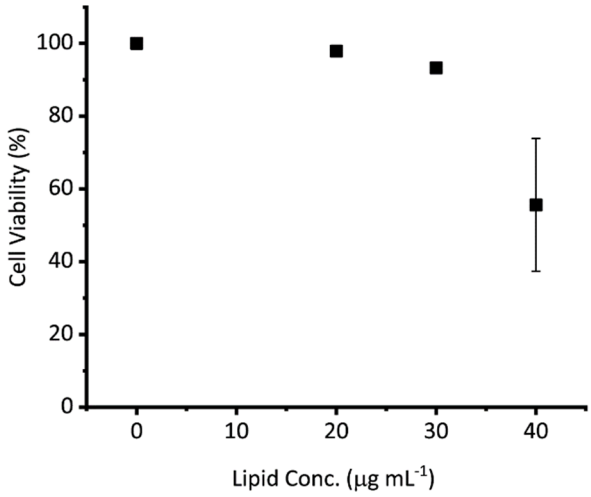

Figure S3. Cell viability of RAW 264.7 cells exposed to increasing amounts of NR_0 for 24 h.

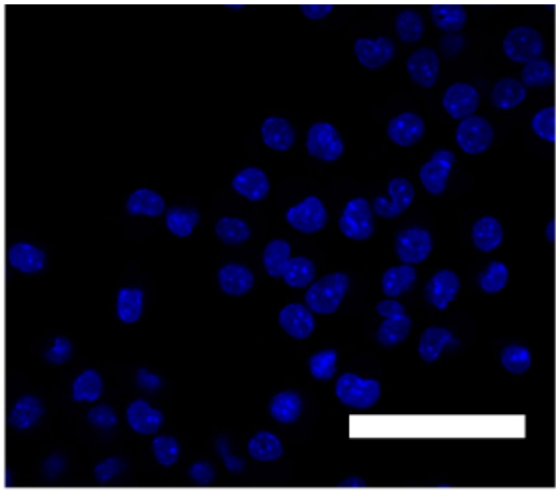

Figure S4. Representative CLSM image of fixed and stained RAW 264.7 cells, confirming the absence of red fluorescence when the cells were not exposed to $^{f}NR_{o}$ (blue: Hoechst stained nuclei). The blue and red channels are overlaid in the same way as in Figure 1d. The scale bar is 50 µm.

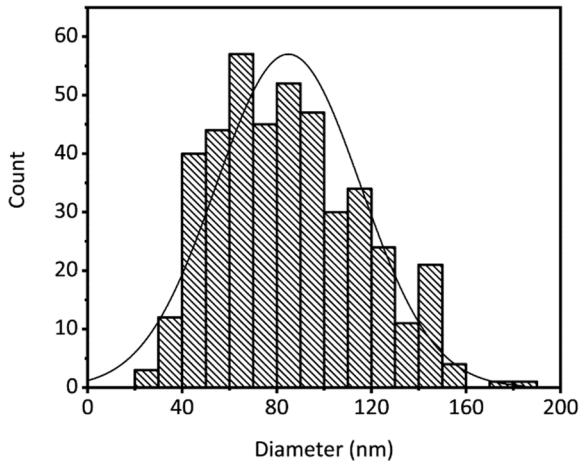

Figure S5. Size histogram of NR_{GOx} as determined from cryo-EM images using the ImageJ software. The histogram and mean value were calculated using Origin software.

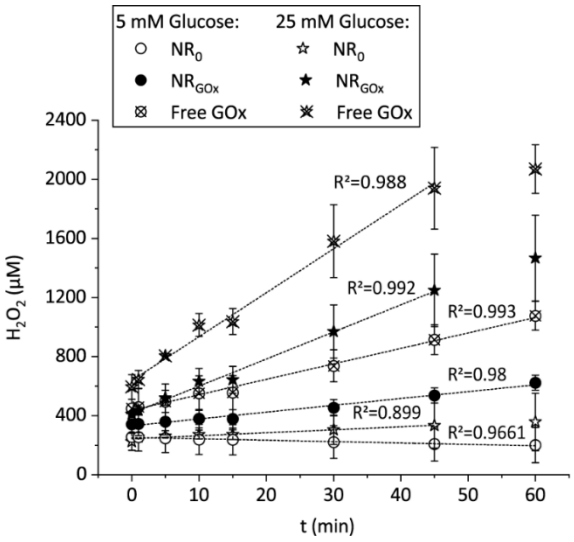

Figure S6. Time-dependent H_2O_2 production in low- (5 mM) and high- (25 mM) glucose media for NR_0, NR_{GOX}, and free GOx. The dashed lines represent the linear fits for each curve.

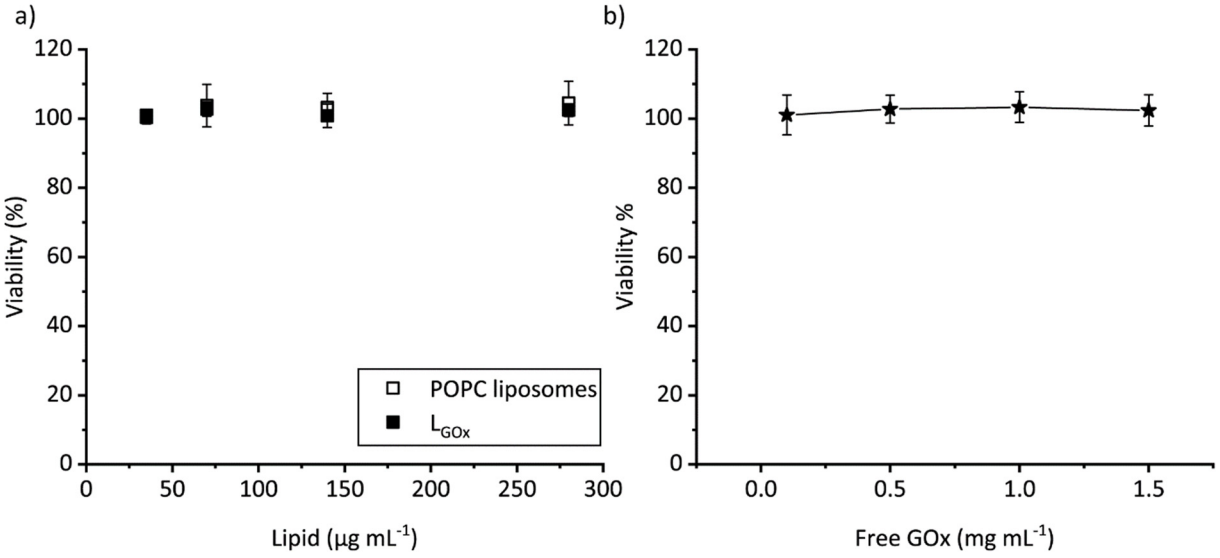

Figure S7. Viability of RAW 264.7 macrophages exposed to glucose-free media for 2 h to a) empty POPC liposomes and GOx-loaded POPC liposomes (L_{GOx}) at concentrations similar to NR_{GOx}, and b) free GOx at concentrations up to 5× higher than the determined encapsulated amount of GOx in NR_{GOx}. Following on, the cells were incubated for 24 h in high-glucose media, and the viability was assessed using the CCK-8 kit. Data represent mean values ± StD (n = 3).

Table S1. N_o, NR_{GOx} and $NR_{\beta\text{-}Gal}$ characterization

Vesicle type	Size (nm)[a)]	PDI[b)]	LE (%)[c)]	Usage
NR_o	206 ± 36	0.20	--	Dosing in RAW 264.7 macrophages
				Controls for $NR_{\beta\text{-}Gal}$ in RAW 264.7 macrophages and primary macrophages
	437 ± 117	0.25	--	Hybrid vesicle imaging
				Controls for the intracellular activity of NR_{Gox} in RAW 264.7 macrophages
NR_{GOx}	445±66	0.27	~35	All the experiments involving NR_{Gox}
L_{GOx}	225±8	0.09	NA	Liposome control experiments
$NR_{\beta\text{-}Gal}$	240 ± 65	0.28	~6	All the experiments involving $NR_{\beta\text{-}Gal}$ beside imaging
	440 ± 97	0.27	N/A	Hybrid vesicle imaging
$L_{\beta\text{-}Gal}$	218 ± 7	0.13	NA	Liposome control experiments

[a)]Hydrodynamic diameter assessed by DLS; [b)]Polydispersity index; [c)]Enzyme loading efficiency

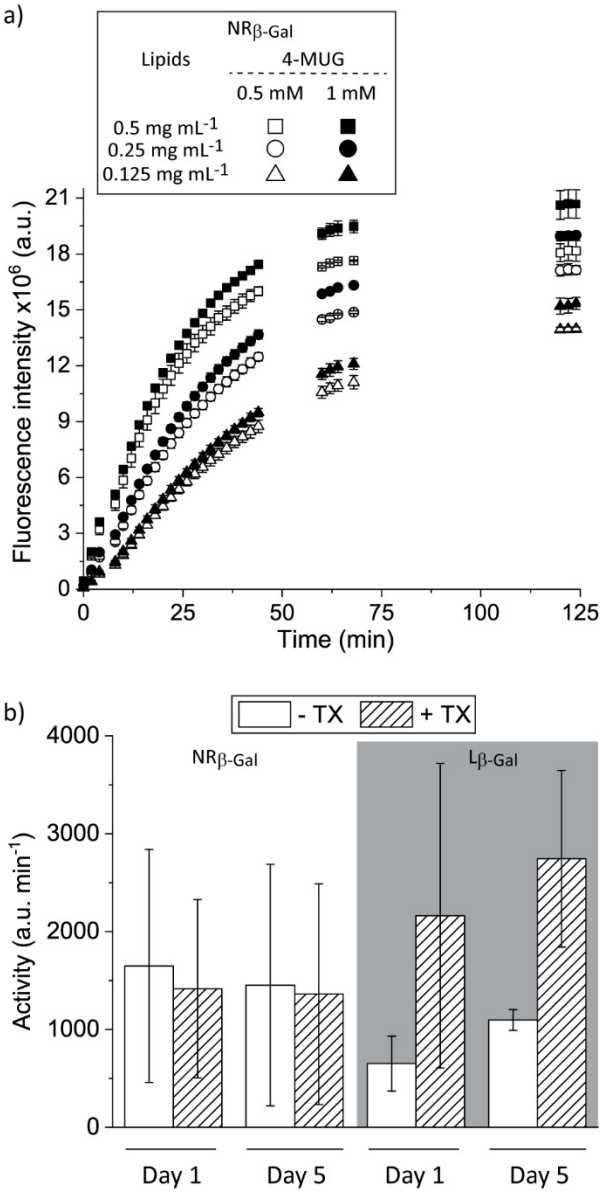

Figure S8. $NR_{\beta\text{-Gal}}$ activity: a) Conversion kinetics of 0.5 mM (open symbols) and 1 mM (closed symbols) 4-MUG into fluorescent 4-MUB by $NR_{\beta\text{-Gal}}$ within 120 min. Three different $NR_{\beta\text{-Gal}}$ concentrations were used. Data represent mean ± StD. (n = 3). b) Slopes obtained from the enzymatic reaction profiles of $NR_{\beta\text{-Gal}}$ and $L_{\beta\text{-Gal}}$ as prepared (day 1) and after 5 days incubation at 37 °C using 0.5 mM 4-MUG in the presence or absence of Triton X (TX 0.1 wt%). $L_{\beta\text{-Gal}}$ represents β-Gal loaded POPC liposomes.

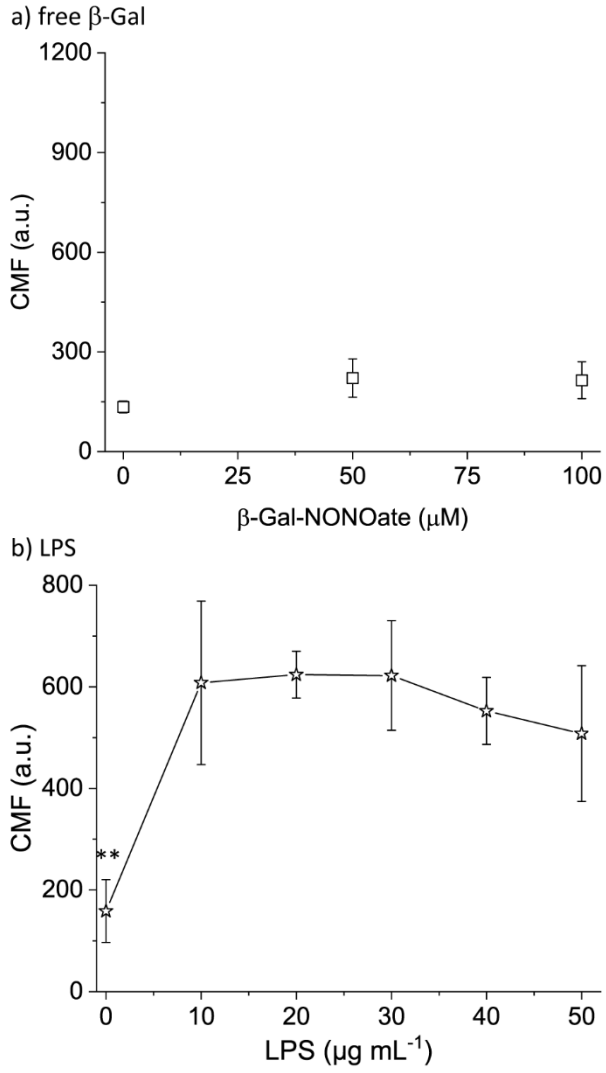

Figure S9. a) CMF of RAW 264.7 cells exposed to β-Gal in solution followed by incubation with different concentrations of β-Gal-NONOate. b) CMF of RAW 264.7 cells stimulated with increasing amounts of LPS for 19 h. In a) and b), the intracellular NO was determined using DAF-FM and flow cytometry as the read-out method. ($n = 3$, $^{**}p < 0.01$)